Evaluating the Biostability of Polypyrrole Microwires

by

Ross J. Wendell

Submitted to the Department of Mechanical Engineering
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Signature of Author_____
Department of Mechanical Engineering
May 8, 2009

Certified by_____
Ian W. Hunter
Hatsopoulos Professor of Mechanical Engineering
Thesis Supervisor

Accepted by_____
John H. Lienhard V
Collins Professor of Mechanical Engineering
Chairman, Undergraduate Thesis Committee

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ABSTRACT

The ability to record signals from the brain has wide reaching applications in medicine and the study of the brain. Currently long term neural recording is precluded by the formation of scar tissue around the electrodes inserted into the brain. Conducting polymers present a possible solution to this problem as their biocompatibility and low stiffness could improve the quality of the interface between the electrode and the brain.

In order to assess the long term stability of conducting polymers, electrodes are fabricated from polypyrrole using a variety of dopants to improve conductivity. These electrodes are then immersed in artificial cerebrospinal fluid while impedance measurements are taken over a period of days.

The impedance of the electrodes increases rapidly for the first 40 hours before leveling off with only a slow increase in impedance being observed over the next 80 hours. When the ends of the electrodes are trimmed the impedance drops and then undergoes an accelerated rise and levels off.

An experiment on the dimensional changes of the polypyrrole reveals that the polymer shrinks when placed into the solution. This may affect the integrity of the electrode and contribute to the increasing impedance. Further research will be necessary to understand the mechanism of the impedance increase and the electromechanical behavior of polymers with different biocompatible dopants.

Thesis Supervisor: Ian W. Hunter
Title: Hatsopoulos Professor of Mechanical Engineering

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1. Introduction

The ability to record signals from the brain has wide reaching applications in medicine and the study of the brain. In recent years a great deal of research has been done on breaking down barriers between the brain and man-made devices such as prosthetics and computers. An ability to record data from neurons for long periods of time would also provide tremendous opportunities for research on the workings of the brain.

The goal of this thesis is to evaluate the biostability of a new class of implantable electrodes. These electrodes, made from conducting polymer, may not suffer from the biocompatibility problems associated with metal electrodes. In order for a material to be suitable for such electrodes it must not degrade electrically or mechanically when implanted and must also not cause death of the surrounding cells. Polypyrrole (PPy) electrodes do not cause cell death and as a result may be excellent candidates for implantation. This thesis will evaluate the suitability of these electrodes from an electrical impedance point of view.

1.1 Electrodes for Neural Recording

In order to gain a better understanding of the brain it is necessary to be able to make accurate recordings of neural activity. This is accomplished with implanted electrodes which can vary greatly in form and material.

Until recently the only suitable method of neural recording involved the use of metal electrodes pressed into the brain. Examples of this type of neural interface include the Utah array (Nordhausen 1996) and Michigan probe (Vetter 2004) both of which are

constructed of silicon. Although electrodes of this type are currently the standard on account of the quality of recordings taken with them, the difference in stiffness between the soft brain tissue and the hard electrodes causes sheer induced inflammation and fibrous encapsulation which interferes with neural recording and eventually renders the metal electrodes useless. However, coating current micromachined neural prosthetic devices with aqueous-based biocompatible polymers has been shown to improve the neuron-implant interface and facilitate high quality acute neural recording (Cui *et al.,* 2002, He *et al.,* 2006, Khan *et al.,* 2007). These coatings must balance biocompatibility with high conductivity which has led to the adoption of doped conducting polymers. These blends of conducting polymers and biomolecules greatly impede the formation of scar tissue and promote cell adhesion to the electrodes (Cui *et al.,* 2001, Massia *et al.,* 2004). These results have led to the consideration of conducting polymers for the construction of the electrodes themselves.

1.2 Conducting Polymers

Most polymers are excellent electrical insulators with conductivities that are orders of magnitude lower than copper. However, a specific class of polymers can be made highly conductive with the inclusion of appropriate dopants. Some of the more common conducting polymers are shown in Figure 1.

All of these polymers possess a conjugated molecular backbone comprised of alternating single and double bonds. The addition of a charged dopant displaces the weakly bound π electrons from the backbone and allows the material to conduct electricity (Anquetil 2004).

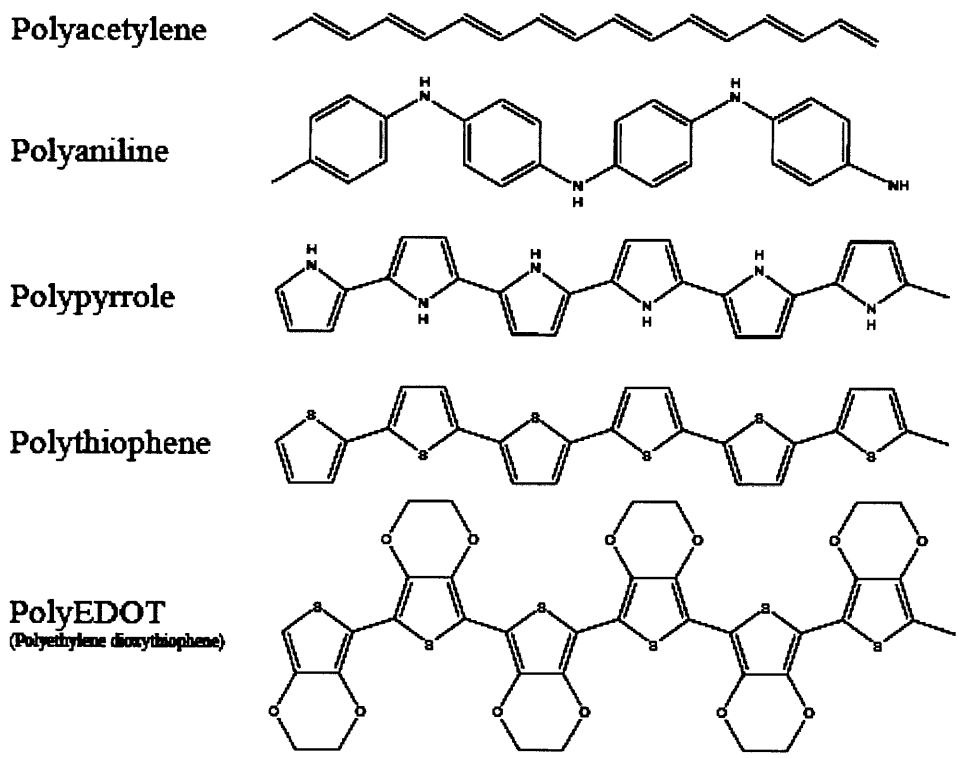

Figure 1: Common conducting polymers showing the alternating single and double bonds. Taken from Anquetil 2004.

Conducting polymer electrodes represent a good candidate for replacing metal electrodes for neural recording. Their biocompatibility and low stiffness (800 MPa; Madden 2000) minimizes the formation of scar tissue which should allow implanted electrodes to gather data over much longer periods of time with less damage to the surrounding tissue than is possible with metal electrodes.

The focus of this thesis will be on polypyrrole because its suitability for electrodes is well established and it is the polymer with which the Bioinstrumentation Laboratory has the most experience.

1.3 The Importance of Impedance

A major concern with conducting polymer electrodes is the stability of their electrical properties when immersed in a solution that differs from the solvent used during the electropolymerization process. Impedance provides a good measure of the electrical properties of a material and as such can be used to evaluate stability. It is measured by running a sinusoidal signal of varying frequency through a circuit and examining the signal that passes through the circuit. The result is divided into two parts, the magnitude of the change in amplitude and the change in phase. The magnitude, measured in ohms, is the equivalent of the resistance for a DC circuit while the phase, measured in degrees, is the offset between the signal that is provided and the signal that passes through the circuit. A suitable electrode will have stable magnitude and phase as well as a sufficiently low magnitude so that signals can be differentiated from background noise.

2. Conducting Polymer Electrode Fabrication

In order to assess the viability of polypyrrole wires for neural recording it is first necessary to fabricate suitable electrodes. This section will cover the production of polypyrrole films and the processing necessary to produce electrodes from the raw film.

2.1 Propylene Carbonate Pyrrole Deposition

Initially, films were deposited using the standard deposition process and chambers. A solution of propylene carbonate (PC) with 0.05 molar tetraethylammonium hexafluorophosphate (TEAP), 0.05 molar pyrrole, and 1.0% V/V water was prepared with vigorous stirring. Nitrogen gas was bubbled through the solution in order to remove any air dissolved in the solution. The deposition setup consisted of a two electrode galvanostatic cell with a cylindrical glassy carbon crucible serving as the working electrode and a sheet of copper with a surface area approximately double that of the working electrode serving as the counter electrode (Figure 2). During the deposition current flowing from the counter electrode to the working electrode will polymerize the pyrrole and deposit it evenly onto the working electrode.

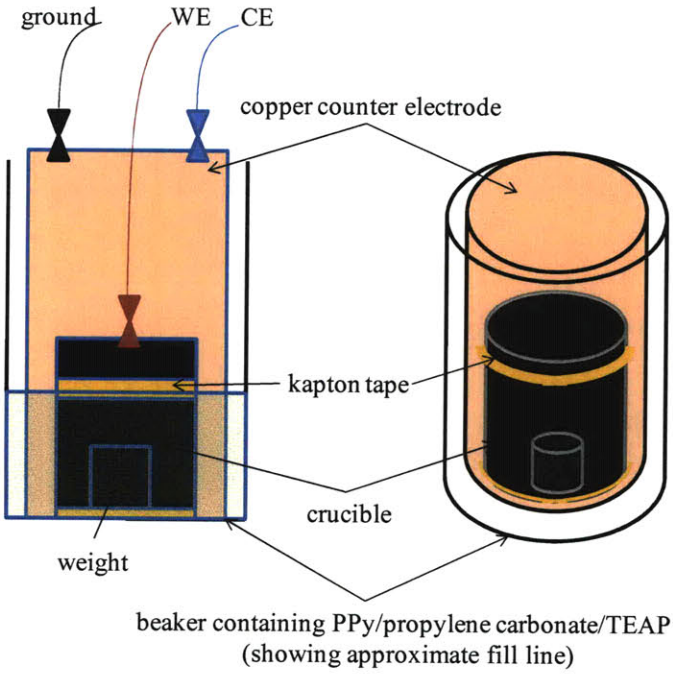

Figure 2: A schematic of the standard electrodeposition setup.

A current of 15.8 milliamps was calculated to achieve a current density of 1.5 A/m^2 at the working electrode. The electrodeposition was performed for 8 hours at -40°C which yielded a glossy black film with a thickness of 20 μm. The film was then rinsed with propylene carbonate and allowed to dry before being removed from the crucible and placed into a resealable plastic bag.

2.2 *Hyaluronic Acid Pyrrole Deposition*

While the standard deposition process yields wires with good conductivity, these wires are not biocompatible. Given that our interest is in generating wires for use as neural electrodes, we have been evaluating the conductivity of polypyrrole films deposited using biocompatible dopant ions (eg. hyaluronic acid [HA], sodium dodecylbenzene sulfonate [DBS], polystyrene sulfonate [PSS] etc) at variable concentration and temperature. Films

exhibiting reasonable conductivity and good biocompatibility (evaluated by neuronal cell growth) have been used to generate wires for evaluation of biostability. The large number of depositions together with the fact that a sufficient number of wires could be generated from a small film resulted in a redesign of the deposition chamber in order to minimize the materials required. A new deposition tank with a volume of 35 milliliters was machined from a block of high density polyethylene (Figure 3).

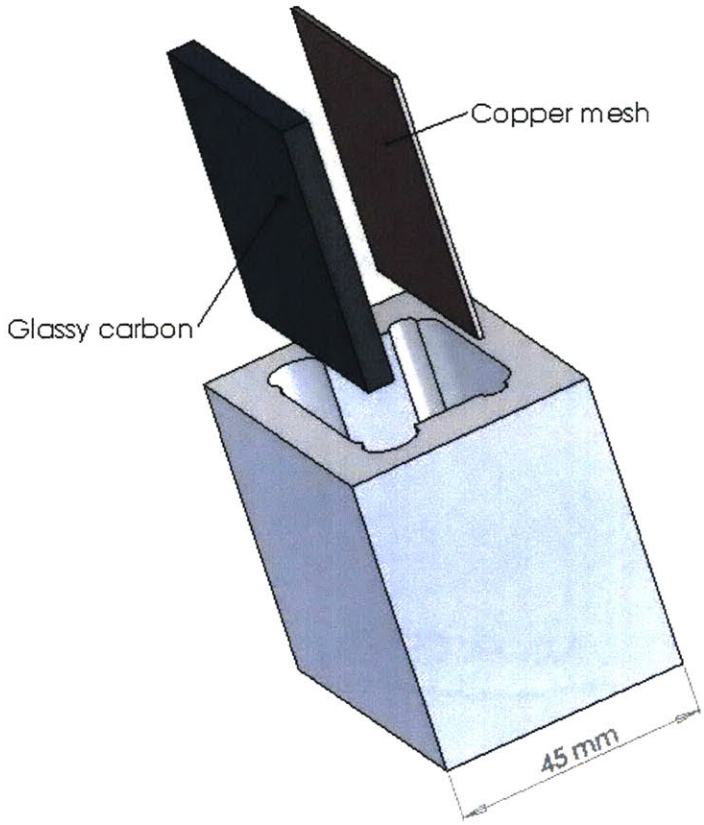

Figure 3: The 35 mL electrodeposition tank used for aqueous depositions.

A solution of 2 mg/mL HA and 0.2 M pyrrole was degassed and sufficient volume poured into the chamber to cover all but a 5 mm strip on the upper edge of a 50 mm square glassy carbon plate (working electrode) placed vertically against one wall of the chamber.

Two sheets of copper mesh (counter electrode) were inserted into the slot on the opposite wall of the chamber. Copper mesh was used instead of copper sheet to increase the surface area of the counter electrode.

The electrodeposition was performed for 6 hours at a temperature of 4°C and current density of 1.5 A/m^2. The film was rinsed with distilled water and allowed to air dry. While drying the film began to curl causing some tearing. However, at least one fragment, with a thickness of 25 μm, was still large enough to be used for wire production.

2.3 Wire Slicing

A piece of polymer film measuring approximately 5 mm by 45 mm was cut with a microtome blade. Distilled water was added to a 50 mm x 20 mm x 20 mm Peel-A-Way® tissue embedding mold (Polysciences, Inc., Warrington, PA) to a depth of approximately 3 mm. The film was floated on the water and the mold was placed at -20°C until the water solidified. Droplets of distilled water were then placed at the corners of the film and frozen. A further 3 mm of distilled water was then added on top of the polymer and frozen, effectively encasing the polymer strip in a block of ice.

In order to cut the film into wires the block of ice was trimmed with a razor blade such that the sides were parallel to the edges of the film. The block was then anchored onto a microtome specimen holder with Optimal Cutting Temperature (O.C.T.) compound (Sakura, Torrance, CA) such that the film was perpendicular to the holder. The holder was mounted in a free-standing ULTRA PRO 5000 cryostat (Vibratome, St. Louis, MO) with the film at a 45 degree angle to the blade cutting surface for propylene carbonate (PPy-PC-TEAP) films and parallel to the blade for aqueous-based films. The 45 degree cutting angle

uses a smaller cutting surface allowing the blade to be horizontally repositioned for continued cutting. However, aqueous films are too brittle when frozen and disintegrate when cut lengthwise. When the aqueous films are cut parallel to the blade almost the entire length of the blade is used for cutting, reducing the number of cuts possible before the blade is dulled. The specimen temperature was set at -15°C for PPy-PC-TEAP films and -3°C for aqueous films while the chamber temperature was maintained at -15°C. The ideal blade angle was 40 degrees for both types of films. The microtome was set to cut sections equal to the thickness of the film in order to produce approximately square wires. The cut wires were removed from the ice with forceps and placed on a lint-free cloth to dry before being placed into a glass vial for storage.

2.4 Electrode Processing

An aluminum fixture was designed and fabricated using a vertical milling machine. It consists of ten stationary clamps and ten clamps that can be moved in the horizontal direction by up to 2 mm. A solid model of the fixture is shown in Figure 4. Five millimeter square silicone rubber pads with a thickness of 3 mm were used on the fixture and the clamps to hold the wires. In order to fixture the wires a thin coating of Dow Corning silicone high vacuum grease was applied to each of the pads (Dow Corning, Midland, MI). This prevented the wires from adhering to the pads during parylene coating and ensured that a 5 mm section would remain uncoated for electrical connections.

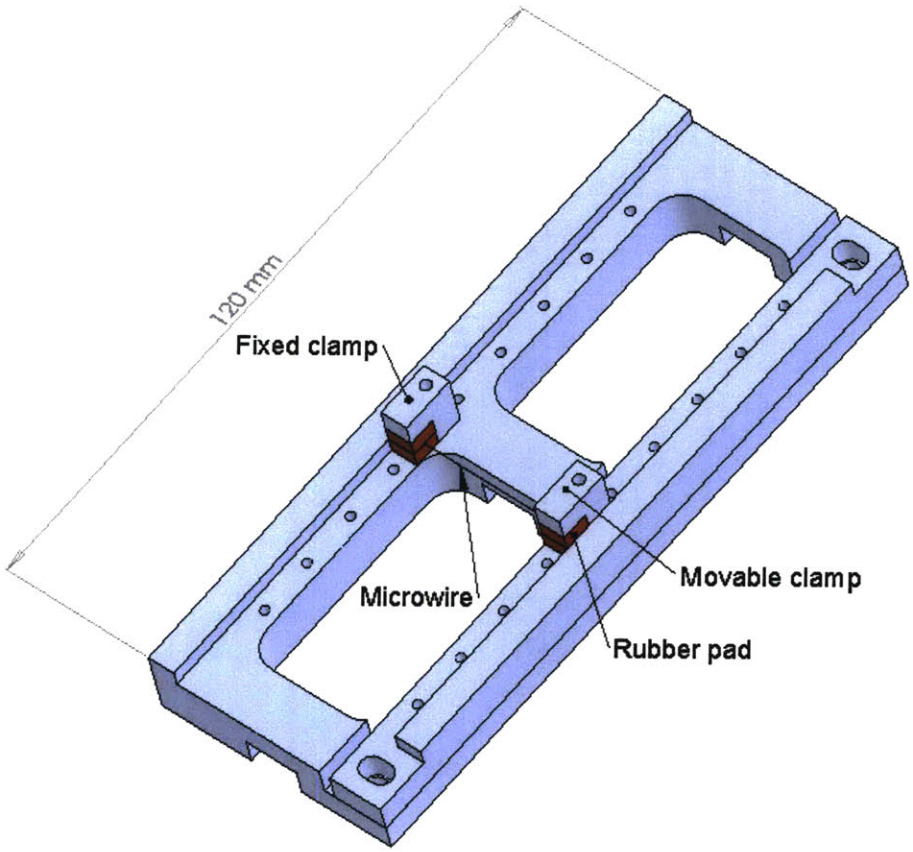

Figure 4: A fixture for holding polymer microwires during processing.

With the movable set of clamps positioned close to the fixed set of clamps one end of each wire was clamped between the pads. Forceps were then used to pull the wire straight while the other end was clamped. Any excess wire protruding from the clamps was cut away with a microtome blade. The movable clamp set was then pulled away from the fixed clamps (maximum of 2 mm) and bolted into place. This ensured that a uniform strain was applied to all ten wires. The fixture was then autoclaved for 10 minutes at 121 °C with a 10 minute drying cycle in order to set the straightness of the wires. Once the fixture had cooled a Para Tech Series 3000 benchtop coating machine (Para Tech, Aliso Viejo, CA) was used to coat the fixture and wires with parylene. Parylene provides an even,

biocompatible, insulating coating. It has a long history of use in biomedical devices with proven safety and stability in implanted devices (Humphrey 1996). Three grams of parylene C dimer were used which yielded a coating thickness of approximately 3 μm. The coated wires were then cut in half with a microtome blade and removed from the clamps yielding 20 electrodes. The electrodes produced in this manner have a 5 mm uncoated section at one end to facilitate electrical connection and an exposed polypyrrole tip at the other end for recording.

3. Impedance Measurement

Evaluating the biostability of polymer microwires in biological fluids will provide an indicator of the feasibility of using the wires as neural electrodes. Impedance measurements were taken by immersing the tip of each electrode in 0.6 mL of artificial cerebrospinal fluid for several days.

3.1 Electrode Immersion

In order to facilitate evaluation of the electrodes in a fluid environment, a set of immersion chambers was designed and fabricated from clear acrylic plastic. Copper sheet was used for contacts and one millimeter thick silicone rubber was used as a gasket. A solid model of an immersion chamber is shown in Figure 5. One contact is isolated from the fluid in the chamber and will make contact with the uncoated end of the electrode. The other contact will be immersed in the fluid with the cut electrode tip to complete the circuit. An electrode is placed with its uncoated end on the isolated contact and the gasket is placed over it. The top is then bolted down and the chamber filled from the fill holes in the top. Once the chamber is full, the fill holes are sealed with nylon bolts and rubber o-rings.

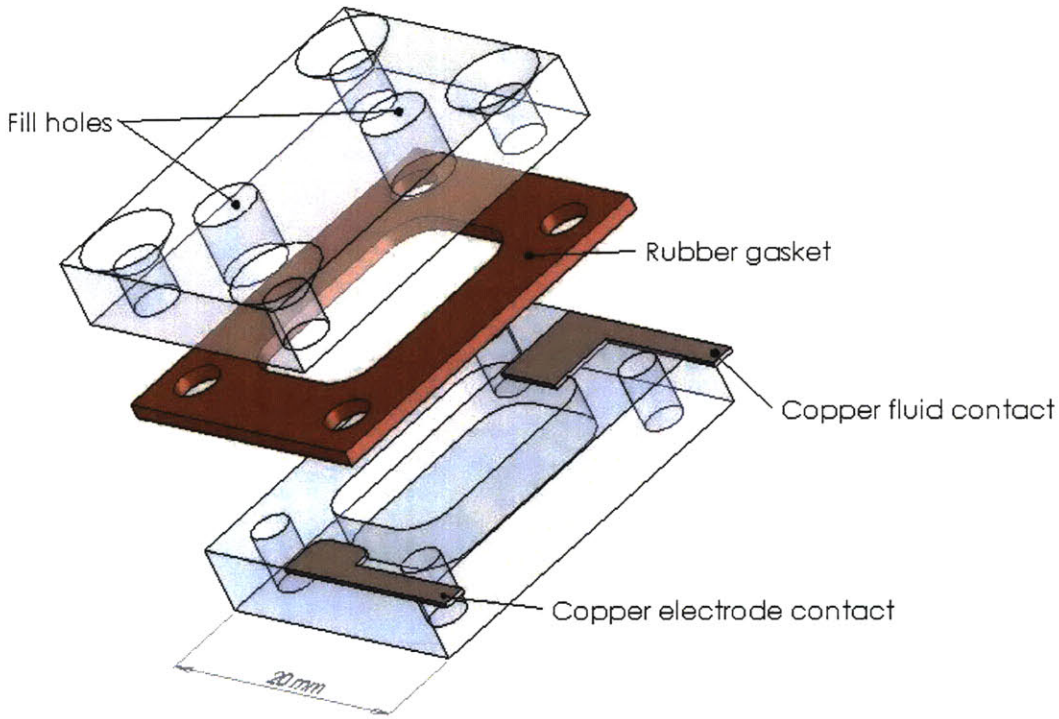

Figure 5: An immersion chamber used to measure electrode impedance in aCSF.

In an effort to evaluate the electrodes under realistic conditions they should be immersed in a fluid similar to what they will be exposed to when implanted in the brain. While the total chamber volume is only 0.6 mL, the total volume of cerebrospinal volume that can be obtained from the atlanto-occipital space of anaesthetized rabbits (our source) is only 1 to 2 mL. As such, we determined that early studies could be done using artificial cerebrospinal fluid (aCSF). aCSF was prepared by combining an equal volume of Solution A (300 mM NaCl, 6 mM KCl, 2.8 mM $CaCl_2$, and 1.6 mM $MgCl_2$) with an equal volume of Solution B (1.6 mM $Na_2HP0_4 \bullet 7H_20$ and 0.4 mM $NaH_2P0_4 \bullet H_20$), pH of 7.3 to 7.4. While CSF obtained from various species contains 22 to 26 mM bicarbonate, bicarbonate

was excluded from the aCSF used in these studies as it can cause a shift in pH as it converts to carbon dioxide and the resultant carbon dioxide can cause bubbles to form within the reaction chamber which would interfere with the electrode-fluid interface.

3.2 Test Setup

In order to gather data for multiple electrodes over a long period of time it was necessary to automate the data collection process. An electronic switching circuit was designed and built by Tommaso Borghi to allow multiple samples to be interfaced to a single Hewlett Packard 4194A impedance analyzer (Agilent Technologies, Santa Clara, CA). The circuit consists of three quad operational amplifiers set up as unity gain buffers and twelve Teledyne 732TN-05 relays (Teledyne Relays, Hawthorne, CA). This circuit allows switching between up to twelve samples.

The impedance analyzer and the switching circuit were interfaced to a computer using National Instruments (NI) LabView software (National Instruments, Austin, TX). The virtual instruments for communicating with the impedance analyzer via a NI GPIB to USB adapter were written by Woong Jin (Chris) Bae (2008) while the switching circuit was controlled directly using a NI DAQPad-6507 digital I/O device. The interface program allows the user to specify the sweep parameters as well as the number of sweeps to perform and the time between each sweep. It first sets the impedance analyzer to a known starting state and then sets the specified sweep parameters. At the specified time intervals it switches on each channel of the switching circuit in succession, performs a frequency sweep and records the magnitude and phase data to a specified output directory. A series of

Matlab (The MathWorks, Natick, MA) scripts were written that query the user for the directory containing the data files of interest and then plot both magnitude and phase.

3.3 Characterizing the Setup

To ensure that the setup would be suitable for evaluating electrode impedance, a series of calibration measurements were obtained which included measuring impedance in the absence of the chambers (switching circuit calibration, Figure 6), in chambers filled with aCSF only (open circuit calibration, Figure 7), and with a tinned copper wire bridging the contacts (closed circuit calibration). Impedance measurements with a tinned copper wire in place of an electrode were also obtained for comparison (Figure 8). The closed circuit calibration measurement was inconclusive because it exceeded the current capabilities of the impedance analyzer. Variation between channels and sweeps is negligible so only single sweep data from the first channel is shown. The Matlab code used to generate these plots is presented in Appendix A.

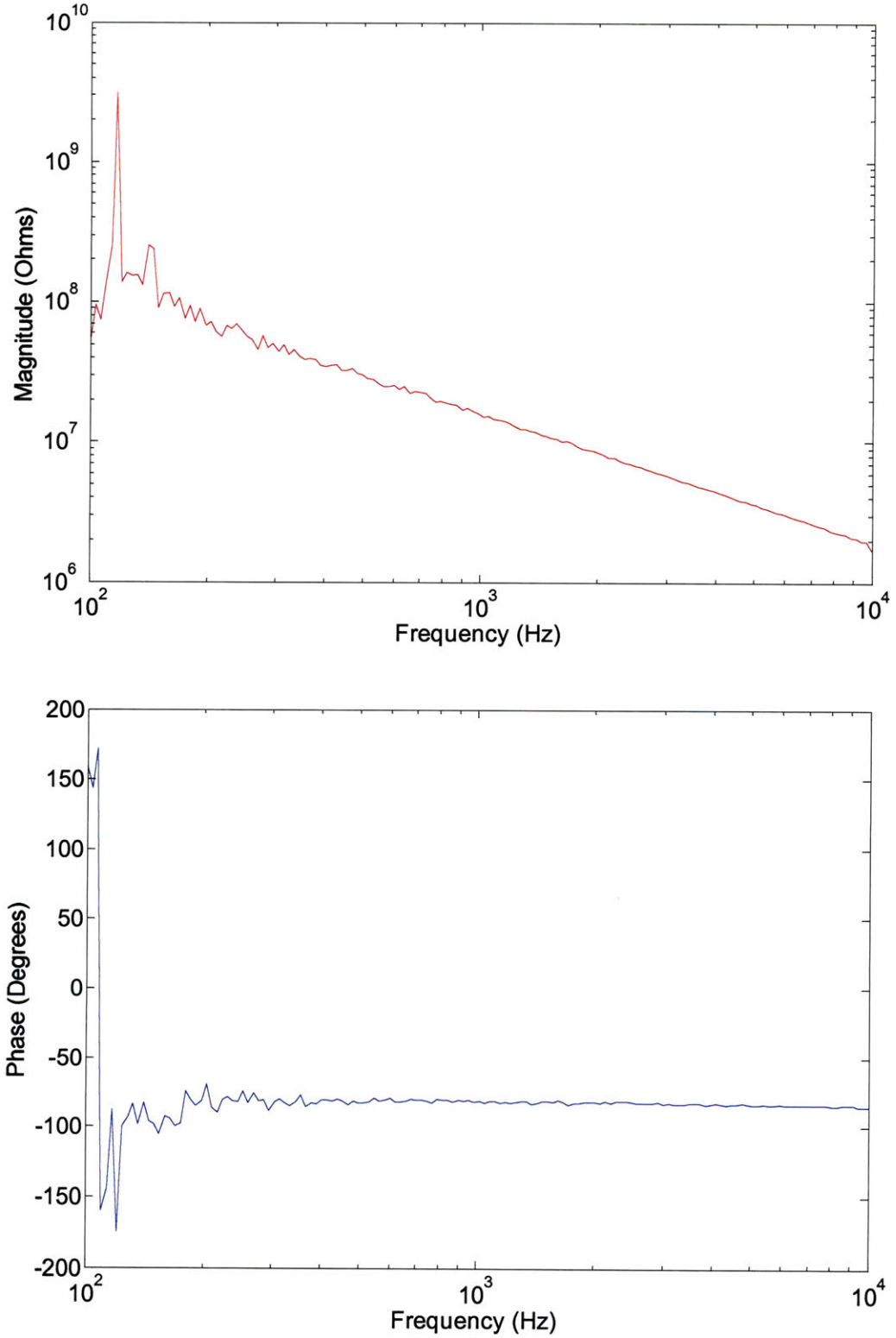

Figure 6: Impedance of the switching circuit with no chamber.

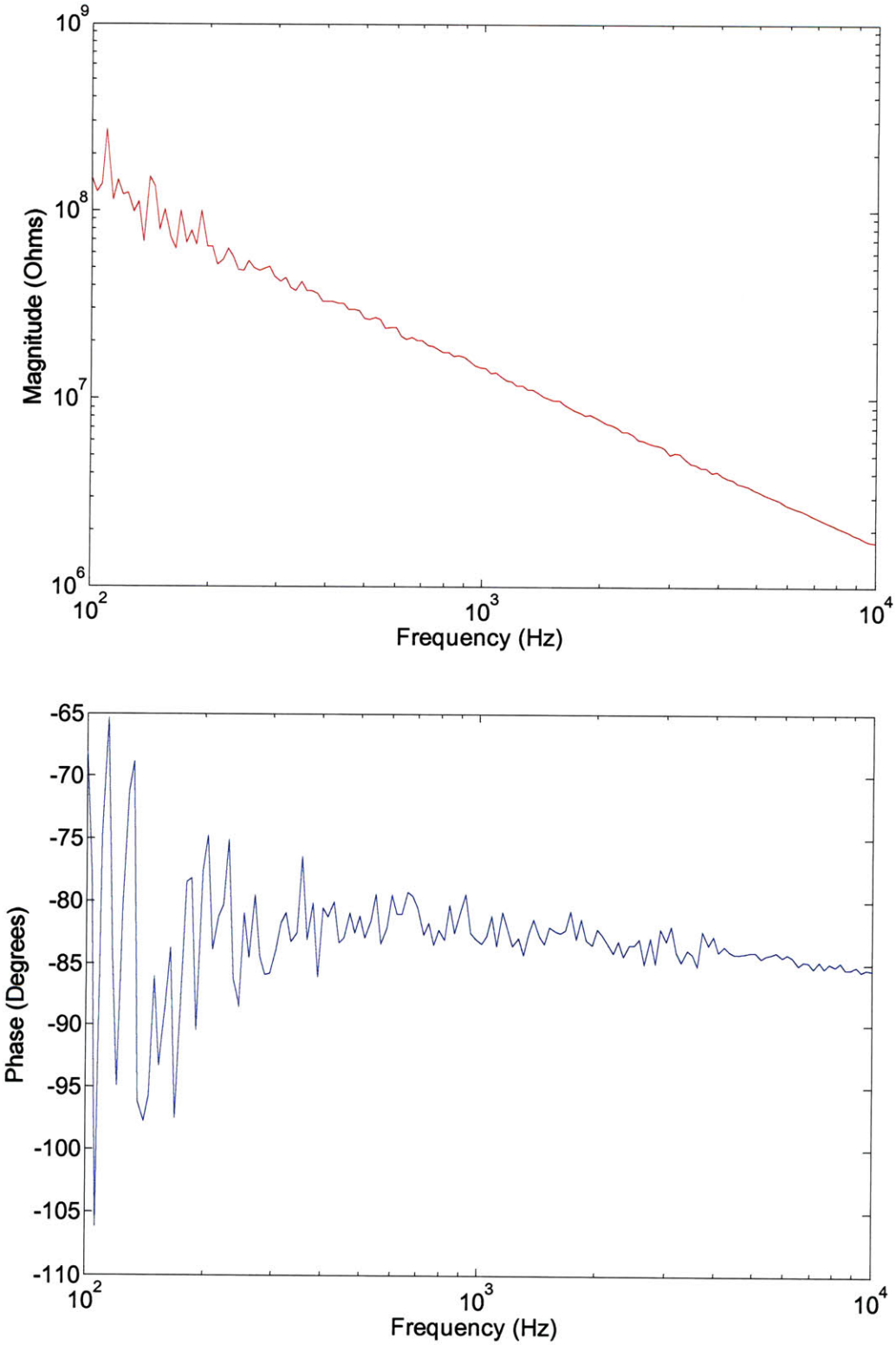

Figure 7: Impedance of a chamber filled with aCSF but no electrode.

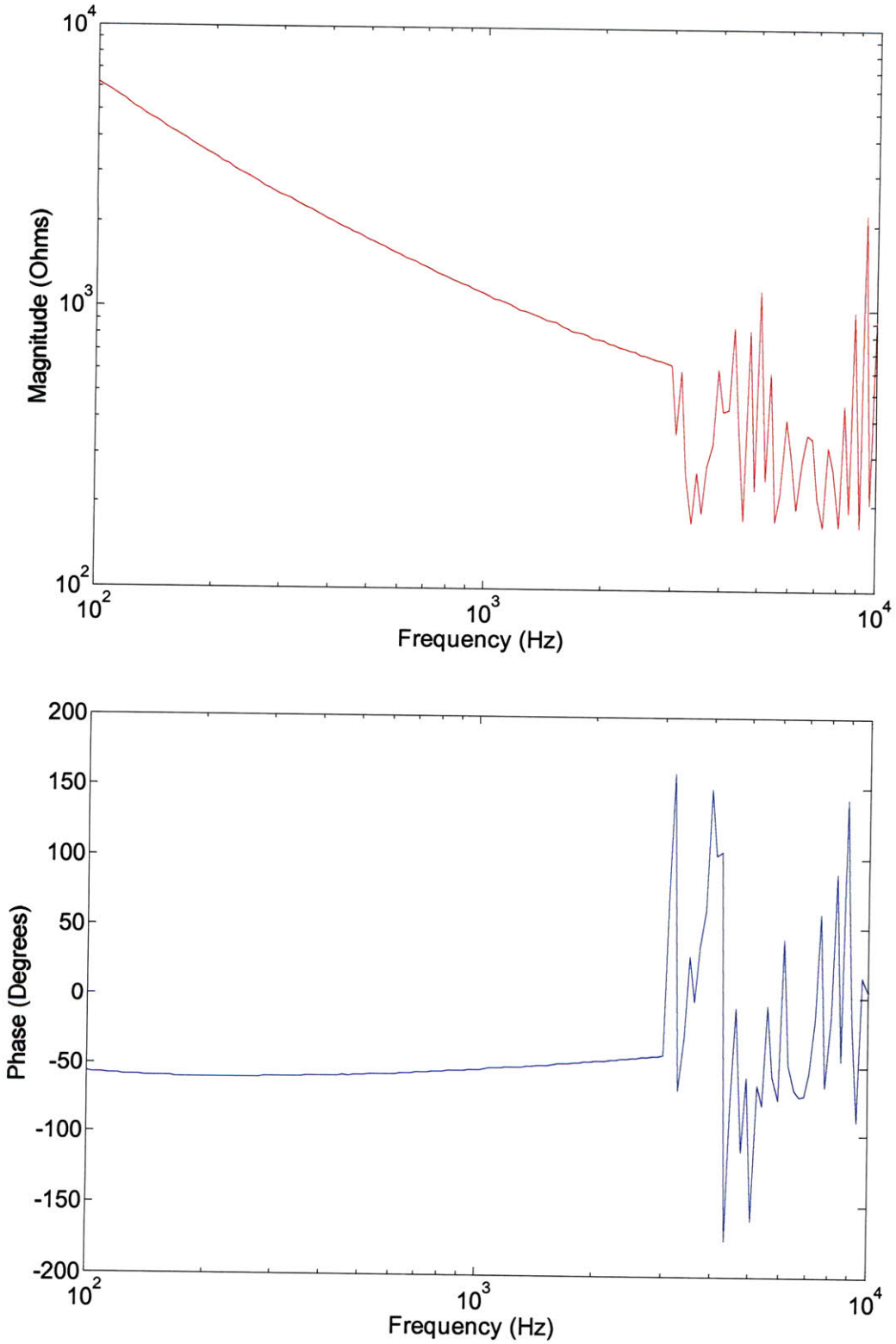

Figure 8: Impedance with a tinned copper wire in place of an electrode.

The impedance ranges from 10^6 ohms at high frequencies to 10^8 ohms at low frequencies for both the system calibration and the open circuit calibration. The similarity between the chamber and no chamber measurements implies that the design of the chamber is suitable for the experiment because its contribution to the impedance of the apparatus is negligible. The wire electrode impedance is quite low (less than 10^4 ohms), although the signal becomes very noisy above 3 kHz, possibly due to outside interference. This setup should be suitable for electrode impedance measurements provided the measured impedance is below the open circuit impedance..

3.4 Microwire Impedance Measurements

Four PPy-PC-TEAP electrodes (referenced as E0...E3) were fixed in individual chambers containing aCSF as discussed in 3.1 Electrode Immersion. A fifth chamber fitted with a tinned copper wire in place of an electrode was included for comparison. The impedance analyzer was set to perform a logarithmic frequency sweep with 150 data points between 100 Hz and 10 kHz. The time between sweeps was set at 60 minutes and the number of sweeps was set at 0 (no limit). The test was stopped after 119 hours but the electrodes were left immersed in the chambers. The impedance of the comparison chamber remained constant demonstrating that the interface between the wire and the aCSF was not changing with time (data not shown). The magnitude and phase data for the four electrodes at 0 and 100 hours are plotted as a function of frequency in Figure 9. The 100 Hz and 10 kHz magnitude and phase data for the four electrodes are plotted as a function of time in Figure 10. A representative plot of the raw data for E3 is included for reference

(Figure 11). The Matlab code used to generate these three types of plots are presented in Appendix B, C, and D respectively.

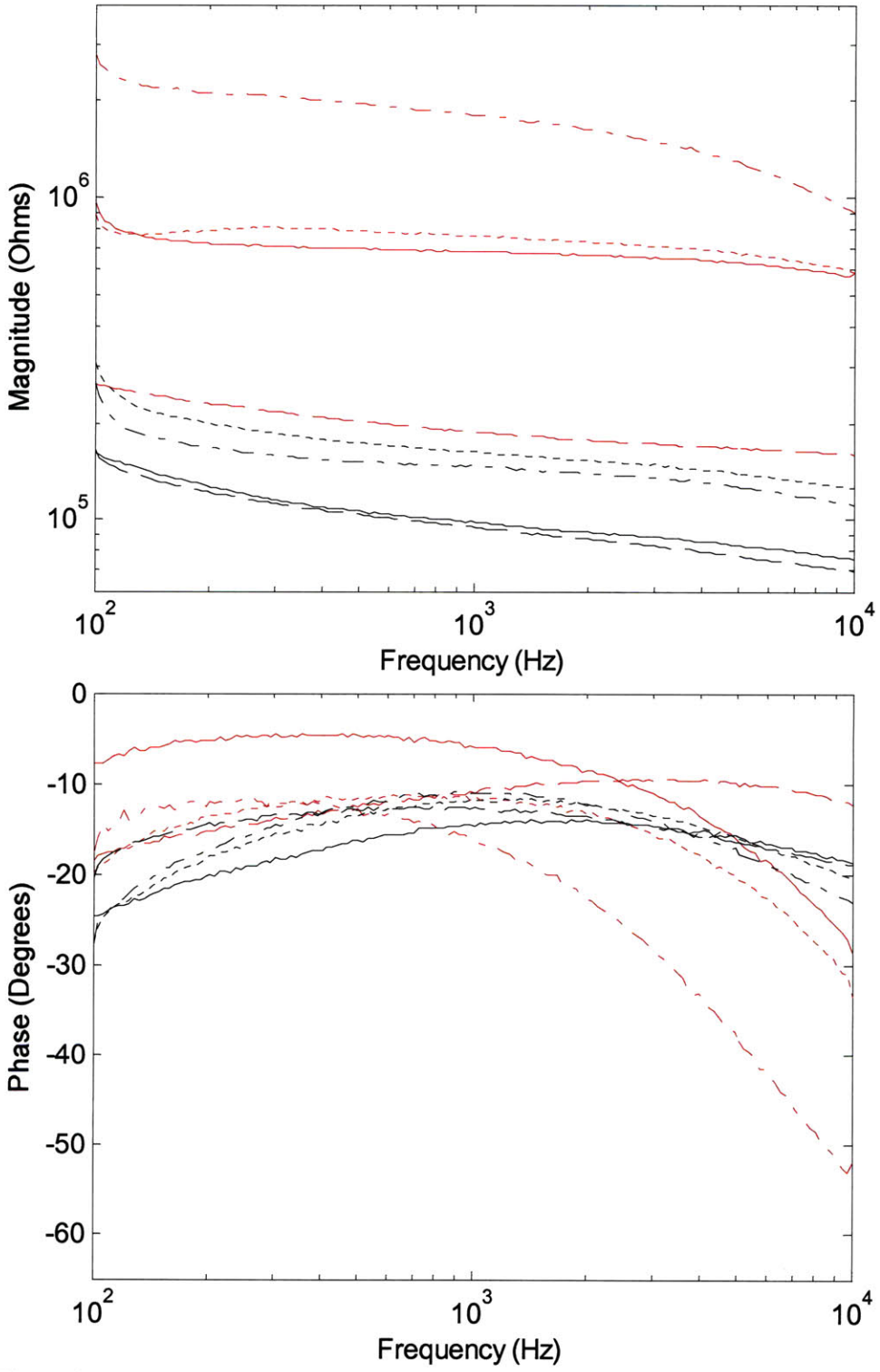

Figure 9: The magnitude and phase data at 0 hours (black) and 100 hours (red) for E0 (solid), E1 (dots), E2 (dashed), and E3(dashed dots).

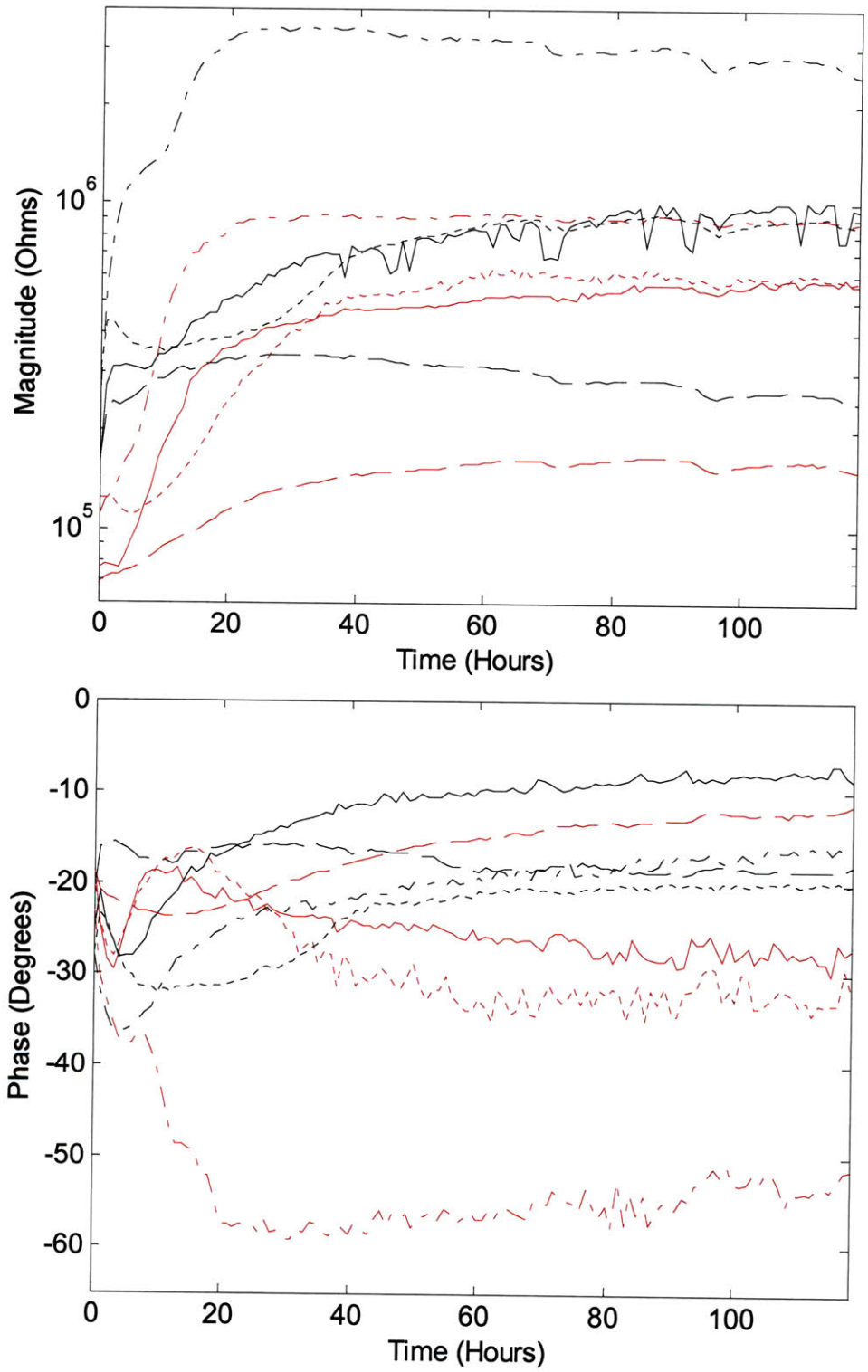

Figure 10: The magnitude and phase data at 100 Hz (black) and 10 kHz (red) for E0 (solid), E1 (dots), E2 (dashed), and E3(dashed dots).

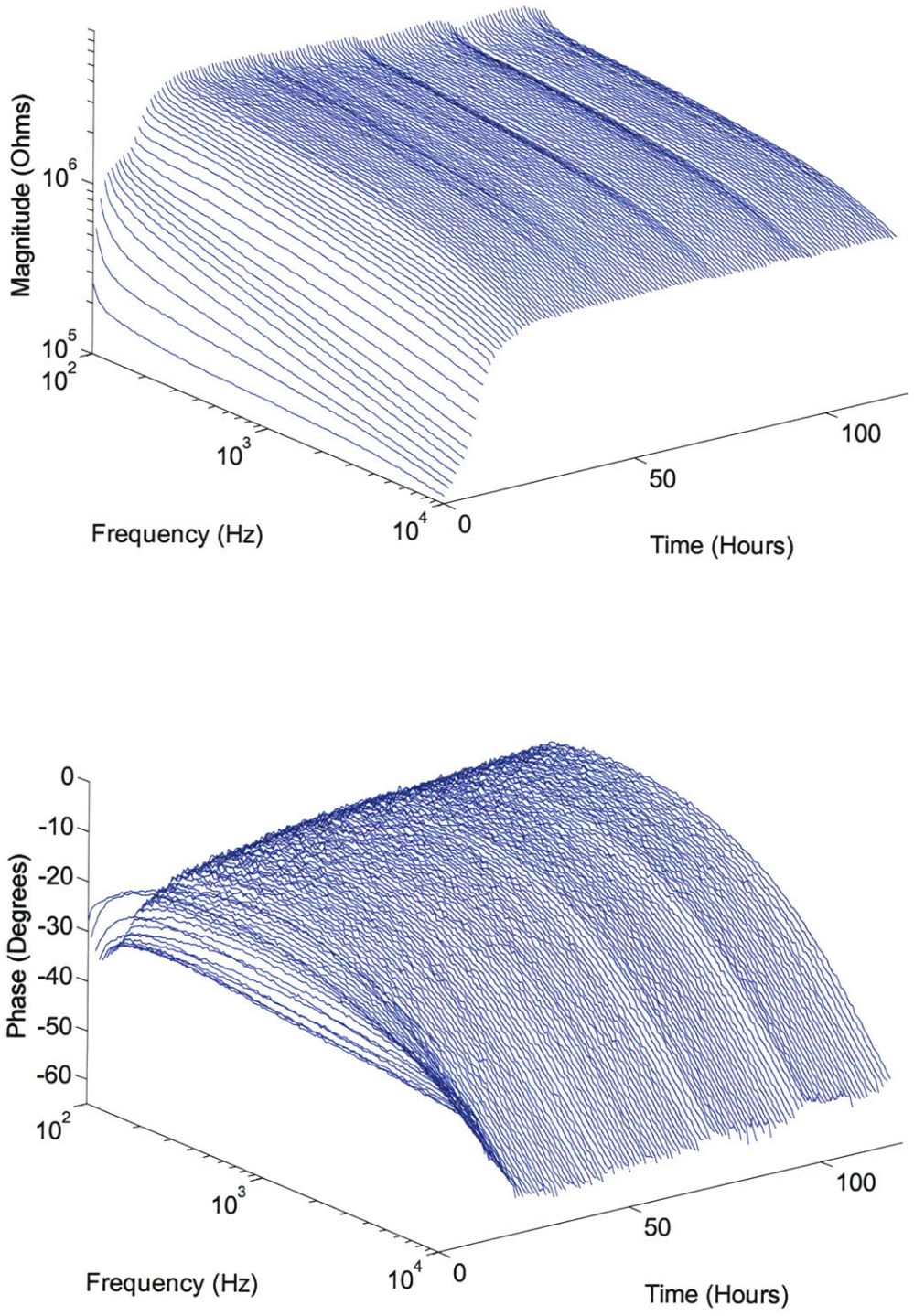

Figure 11: Raw impedance data for E3 for 119 hours following initial immersion in aCSF.

The values for magnitude and phase vary significantly between the electrodes possibly due to the ~1 mm variation in the length of the electrodes. The largest discrepancy is visible in the data collected for E2 where the magnitude and phase show little change over time; the wire itself, the manner in which it is seated in the fixture, and/or the fixture itself and the connects may be problematic. However, the other three wires clearly display a general, albeit variable, change in the impedance over time. The impedance changes dramatically in the first 25 hours and then begins to level off, after which one observes a slow increase in the magnitude and marginal change in the shape of the phase curve. The final impedance falls in the range of 0.6 to 2 megaohms with the impedance remaining relatively constant from 200 Hz to 10 kHz. It should be noted that at frequencies approaching 10 kHz the impedance measured is very close to the open circuit impedance. This implies that the testing setup may not be suitable for measuring the electrode impedance at high frequencies.

In addition, a slight drop in the impedance was observed in all samples approximately every 24 hours. This could reflect a change in fluid temperature caused by a change in the ambient temperature of the laboratory with a nightly drop being evidenced in the data.

3.5 Electrode Tip Clipping

After a further 2 weeks of immersion another set of data was taken for the same electrodes using the same sweep parameters as before. After establishing a new baseline impedance the chambers were drained and opened with an effort being made not to disturb the position of the electrode on the copper contact. Each electrode was laid on a steel ruler

and approximately 1.5 mm was cut off the tip with a microtome blade. The chambers were then resealed and refilled with aCSF. Data collection was allowed to continue uninterrupted for a further 122 hours. The goal of this experiment was to better understand the cause of the impedance changes that are observed when the electrodes are immersed in aCSF. The magnitude and phase data at 100 Hz and 10 kHz for the four electrodes are shown in Figure 12.

The initial impedance is similar to the impedance of the electrodes observed after 119 hours of immersion although the magnitude is slightly higher, in line with the slow increase observed in the initial data. The sharp change in magnitude and phase at ~40 hours corresponds to the cutting of the electrode tips. There is some recovery in both magnitude and phase followed by a continued slow increase in magnitude. The phase becomes almost constant across the frequency spectrum after cutting, although the shape of the magnitude curve varies greatly between the electrodes.

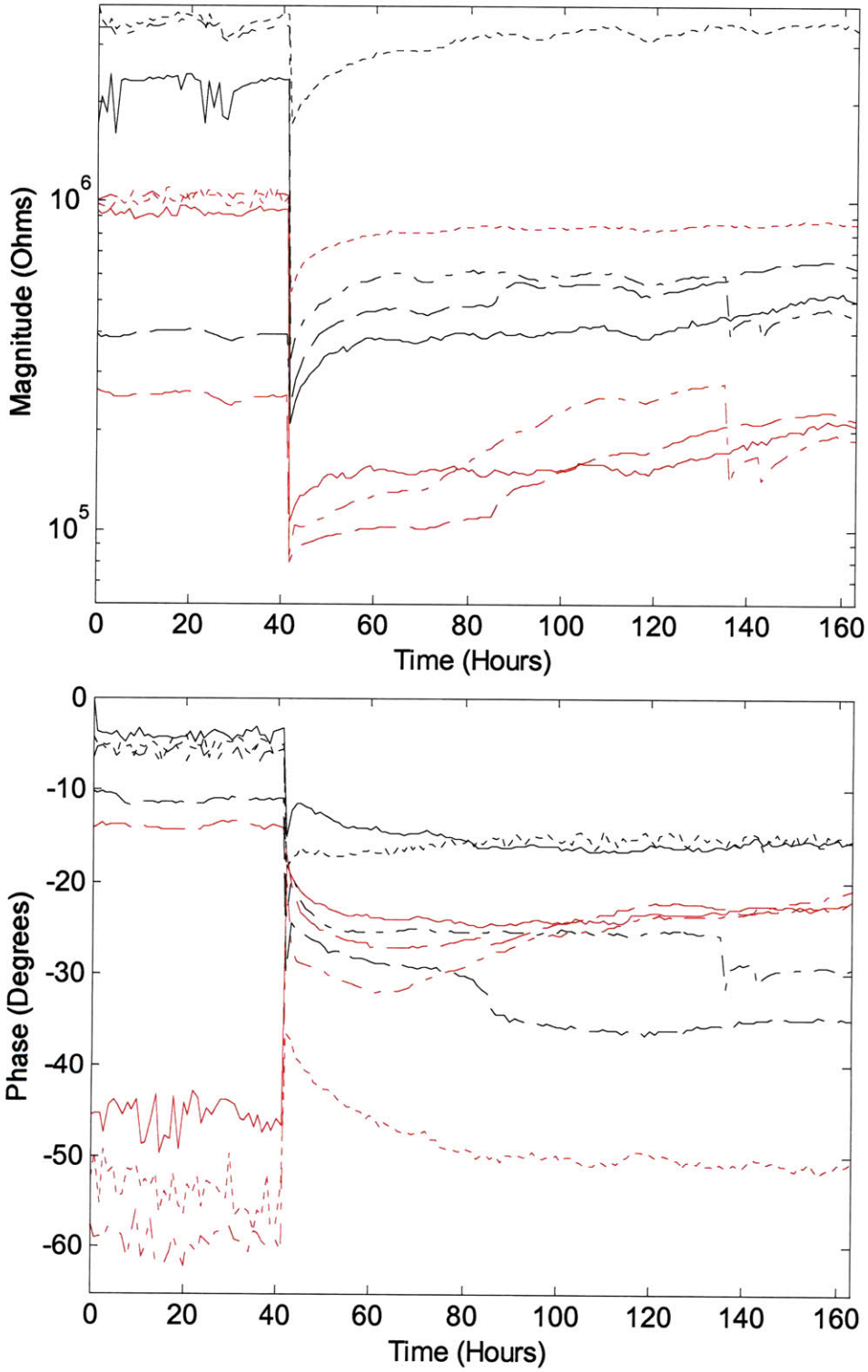

Figure 12: Impedance data at 100 Hz (black) and 10 kHz (red) before and after electrode tip cutting for E0 (solid), E1 (dots), E2 (dashed), and E3(dashed dots).

3.6 Discussion

There are two likely explanations for the observed change in impedance. The first is that the change is solely a result of long range diffusion. As the PC diffuses out of the electrode and the ions in the aCSF diffuse in, the electrical properties of the polymer may change. The rapid change observed when the electrodes are first immersed followed by a leveling off after a period of time could be explained by rapid diffusion of ions near the electrode-fluid interface followed by slower diffusion down the length of the wire, analogous to the evolution of the temperature profile in an infinite rod with a constant tip temperature. When the tip is cut off a new section of the electrode is exposed directly to the fluid and more rapid diffusion takes place at the new interface, followed once again by slower diffusion along the length of the wire. This would explain the initial decrease in impedance. The new tip will behave like a tip that has been immersed for less time. However, diffusion will cause the impedance to change again, resulting in the slow increase in impedance observed after cutting.

A second possible explanation is a combination of short range diffusion and physical damage. If immersion in aCSF causes the polymer to change in size it could damage the interface between the polypyrrole and the parylene sheath, greatly increasing the volume of electrode exposed to the fluid (Figure 13). Thus even if the effects of diffusion are limited to the surface of the polymer when the tip is cut off the recovery will be small because the tip is no longer the dominant electrode-fluid interface. More research will be needed to determine which of these effects, if either, is the dominant cause of the observed impedance change.

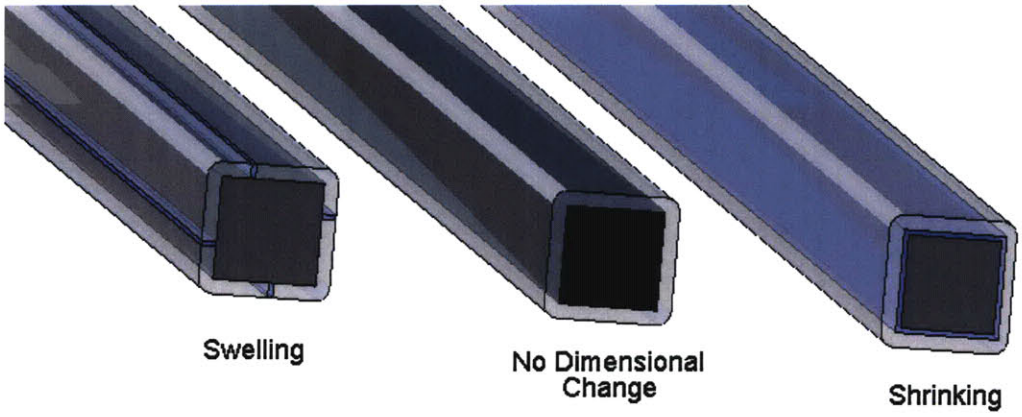

Figure 13: Possible dimensional changes in the PPy and their effect on the PPy-parylene interface.

4. Dimensional Change of Wires in aCSF

In order to determine if dimensional change and damage are a significant factor in the observed change in the impedance of the electrodes, a strip of raw PPy-PC-TEAP was measured prior to immersion in aCSF. Dimensional changes were quantified as a change in the length of the PPy-PC-TEAP strip because that was the largest dimension and thus any change would be more pronounced in that dimension..

4.1 Experimental Setup

A strip of PPy-PC-TEAP film, 5 mm wide, 95.75 mm long, and 20 μm thick was cut from a raw film using a microtome blade. A 130 mm length of Tygon tubing was cleaned with soap and rinsed with tap water followed by three rinses with distilled water. One end of the tube was plugged with a rubber stopper and the tube was filled with aCSF. The strip was placed in the tube and the open end plugged with a rubber stopper. The tube was gently inverted until the film spread out in the tube and most of the air bubbles had collected at one end. The tube was then supported such that one end was higher than the other, allowing any air bubbles that might form to collect away from the film.

4.2 Results and Significance

After 120 hours of immersion, the strip of film was removed from the aCSF and immediately measured. The length of the strip was measured to be 95.25 mm. This corresponds to a 0.5 % decrease in the length. After 15 minutes of drying the film had further decreased in length to 92.0 mm. This is a shrinkage of almost 4% from the original length of the strip. This phenomenon could be explained by the diffusion of PC out of the film. The ions in the aCSF are significantly smaller than PC and thus the replacement of PC ions with ions from the aCSF would cause the film to shrink. This shrinking could potentially be causing the electrode to pull away from the parylene insulation, allowing fluid to move along the length of the electrode by capillary action. Further research on this dimensional change and its affect on the integrity of the electrode is required before making any definitive conclusions.

5. Conclusions and Future Work

After an initial equilibration period, polypyrrole electrodes show promise for long term neural recording. The impedance does continue to change over the long term, but the change is relatively slow and could probably be accounted for when using the electrodes. Of greater concern is the biocompatibility of the electrodes themselves. While this thesis has evaluated the change in impedance associated with prolonged exposure of PPy-PC-TEAP microwires to aCSF, both propylene carbonate and TEAP are known irritants and as such should not be used to generate electrodes for implantation. With this in mind, focus has shifted toward the evaluation and optimization of parameters required to generate stable, conductive, aqueous-based polypyrrole films using biocompatible dopant ions. More specifically, PPy-HA films have been generated and shown to support neuronal cell growth. In the near future, the biostability of microwires obtained from these films will be evaluated using the hardware described in this thesis.

In addition to long term impedance measurements it is our intent to examine the mechanical properties of these microwires for comparison with the wires generated from PPy-PC-TEAP films. Furthermore, the morphology of both sets of wires prior to and post immersion in aCSF will be assessed by Scanning Electron Microscopy (SEM) to determine if the parylene is detaching from the wires over the course of the study and/or if the tip morphology changes after immersion in aCSF and again after tip clipping. The actual shape of the tips will also be evaluated, the thought being that tip geometry may be an important determinant in variable impedance measures between wires. Finally the variable

length of the wires will be addressed with the use of a wire cutting fixture that can be attached to the coating fixture and allow a microtome blade to be precisely and repeatably positioned to cut the wires.

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Appendix A

```
% Impedance plotter
% Ross Wendell 3-29-2009
%
% This script parses all the impedance data files in a directory and
plots
% magnitude and phase
% Start:100 Stop:10000 #Points:150

clear all                                                % Housekeeping
clc
figure(1)
clf
figure(2)
clf

HomeDirectory = 'I:\';                                   % MATLAB Home
Directory
DataDirectory = input('Data directory?\n','s');          % Query for data file
subdirectory
FileExtension = '.txt';                                  % Extension used for
data files
delimiter = '\t';                                        % data file delimiter
R=0;                                                     % start row
C=1;                                                     % start column

cd(HomeDirectory)                          % Make sure that we are in the
home directory
cd(DataDirectory)                          % Navigate to the directory of
data files
files = dir;                               % Retrieve a structured list of
filenames
for m = 1:length(files)                    % Main loop - cycle through all
the files
    filename = files(m).name;              % Extract the current filename
from the list
    if isempty(strfind(filename,FileExtension)) % Is it a text file?
        continue                           % If not, skip it.
    end
    try
        impdata = dlmread(filename, delimiter, R, C);     % Read in a
matrix of data from the file
    catch ME
        continue                           % If that didn't work, skip the
file
    end
    N = length(impdata);
    if N < 150                              % Is the file too short to
contain data?
        OutStr = [10 filename ' is not a full data set']% If so, note
that fact
```

```
        continue                            % Don't try to process the file,
and move on
    end
    freq = impdata(:,1);                    % Extract the frequency data
    Magnitude = impdata(:,2);               % Extract the magnitude data
    Phase = impdata(:,3);                   % Extract the phase data
    figure(1)                               % Make Figure 1 active figure
    set(figure(1),'Position',[10,10,1260,940])     %set position
    ylim([1000000 1000000000])                  % set Y axis limits
    loglog(freq,Magnitude)                % plot magnitude with semilog
scale
    xlabel('Frequency (Hz)')
    ylabel('Magnitude (Ohms)')
    set(gcf,'Color','w')
    hold on                                 % plot all curves together
    figure(2)                               % Make Figure 2 active
    set(figure(2),'Position',[1290,10,1260,940])
    ylim([-90 0])
    semilogx(freq,Phase)
    xlabel('Frequency (Hz)')
    ylabel('Phase (Degrees)')
    set(gcf,'Color','w')
    hold on
    pause(0.25)                             % Wait 0.25 seconds between
curves
end
cd(HomeDirectory)
```

Appendix B

```
% Frequency Snapshop plotter
% Ross Wendell 5-7-2009
%
% This script parses all the impedance data files in a directory and
plots
% 0 and 100 hour magnitude and phase for all electrodes


clear all                                                  % Housekeeping
clc
figure(1)
clf
figure(2)
clf

HomeDirectory = 'C:\Impedance';                                  % MATLAB
Home Directory
DataDirectory = input('Data directory?\n','s');     % Query for data file
subdirectory
FileExtension = '.txt';                                    % Extension used for
data files
delimiter = '\t';                                          % data file delimiter
R=0;                                                       % start row
C=1;                                                       % start column
MagnitudeOut=[];
PhaseOut=[];

cd(HomeDirectory)                            % Make sure that we are in the
home directory
cd(DataDirectory)                               % Navigate to the directory
of data files
folders=dir;
for m = 3:length(folders)                     % Main loop - cycle through
all the folders
    foldername = folders(m).name;               % Extract the current folder
name from the list
    cd(foldername)                              % navigate to the current
folder
    files = dir;                                % Retrieve a structured list
of filenames
    for m = 3:103:length(files)                     % file loop - cycle
through initial and 100 hour impedance data
        filename = files(m).name;               % Extract the current
filename from the list
        if isempty(strfind(filename,FileExtension)) % Is it a text file?
            continue                            % If not, skip it.
        end
        try
            impdata = dlmread(filename, delimiter, R, C);     % Read in a
matrix of data from the file
```

```
        catch ME
            continue                        % If that didn't work, skip
the file
        end
        N = length(impdata);
        if N < 150                           % Is the file too short to
contain data?
            OutStr = [10 filename ' is not a full data set']% If so, note
that fact
            continue                        % Don't try to process the
file, and move on
        end
        freq = impdata(:,1);                % Extract the frequency data
        Magnitude = impdata(:,2);           % Extract the magnitude data
        Phase = impdata(:,3);               % Extract the phase data
        MagnitudeOut = [MagnitudeOut Magnitude];
        PhaseOut = [PhaseOut Phase];
    end
    cd(HomeDirectory)
    cd(DataDirectory)
end
figure(1)                               % Make Figure 1 active figure
set(gcf,'DefaultAxesColorOrder',[0 0 0;1 0 0])
set(0,'DefaultAxesLineStyleOrder',{'-',':','--','-.'})
loglog(freq,MagnitudeOut)                % plot magnitude with semilog scale
xlabel('Frequency (Hz)')
ylabel('Magnitude (Ohms)')
set(gca,'XScale','log','YScale','log')
set(gcf,'Color','w')
xlim([100 10000])
ylim([60000 4000000])                   % set Y axis limits
figure(2)                               % Make Figure 2 active
set(gcf,'DefaultAxesColorOrder',[0 0 0;1 0 0])
set(0,'DefaultAxesLineStyleOrder',{'-',':','--','-.'})
plot(freq,PhaseOut)
xlabel('Frequency (Hz)')
ylabel('Phase (Degrees)')
set(gca,'XScale','log')
set(gcf,'Color','w')
ylim([-65 0])
xlim([100 10000])
cd(HomeDirectory)
```

Appendix C

```
% Time Snapshop plotter
% Ross Wendell 5-7-2009
%
% This script parses all the impedance data files in a directory and
plots
% 100 and 10000 Hz magnitude and phase as a function of time


clear all                                                % Housekeeping
clc
figure(1)
clf
figure(2)
clf

HomeDirectory = 'C:\Impedance';                                  % MATLAB
Home Directory
DataDirectory = input('Data directory?\n','s');      % Query for data file
subdirectory
x = input('Number of samples?\n');
y = input('Number of sweeps?\n');
FileExtension = '.txt';                                  % Extension used for
data files
delimiter = '\t';                                        % data file delimiter
R=0;                                                     % start row
C=1;                                                     % start column
freq100out = 0*ones(x,y);                  % Extract the frequency data
Magnitude100out = 0*ones(x,y);             % Extract the magnitude data
Phase100out = 0*ones(x,y);                 % Extract the phase data
freq1kout = 0*ones(x,y);
Magnitude1kout = 0*ones(x,y);
Phase1kout = 0*ones(x,y);
freq10kout = 0*ones(x,y);
Magnitude10kout = 0*ones(x,y);
Phase10kout = 0*ones(x,y);


cd(HomeDirectory)                             % Make sure that we are in the
home directory
cd(DataDirectory)                               % Navigate to the directory
of data files
folders=dir;
for m = 3:length(folders)                      % Main loop - cycle through
all the folders
    foldername = folders(m).name;               % Extract the current folder
name from the list
    cd(foldername)                              % navigate to the current
folder
    files = dir;                                % Retrieve a structured list
of filenames
```

```
    t=0;
    for n = 3:length(files)                    % file loop - cycle through
initial and 100 hour impedance data
        filename = files(n).name;              % Extract the current
filename from the list
        if isempty(strfind(filename,FileExtension)) % Is it a text file?
            continue                           % If not, skip it.
        end
        try
            impdata = dlmread(filename, delimiter, R, C);     % Read in a
matrix of data from the file
        catch ME
            continue                           % If that didn't work, skip
the file
        end
        N = length(impdata);
        if N < 150                              % Is the file too short to
contain data?
            OutStr = [10 filename ' is not a full data set']% If so, note
that fact
            continue                           % Don't try to process the
file, and move on
        end
        Magnitude100 = impdata(1,2);             % Extract the magnitude
data
        Phase100 = impdata(1,3);                 % Extract the phase data
        Magnitude1k = impdata(76,2);
        Phase1k = impdata(76,3);
        Magnitude10k = impdata(150,2);
        Phase10k = impdata(150,3);

        Magnitude100out(m-2,n-2) = Magnitude100;        % append the
magnitude data
        Phase100out(m-2,n-2) = Phase100;                % append the
phase data
        Magnitude10kout(m-2,n-2) = Magnitude10k;
        Phase10kout(m-2,n-2) = Phase10k;
    end
    cd(HomeDirectory)
    cd(DataDirectory)
end
figure(1)                                  % Make Figure 1 active figure
set(0,'DefaultAxesLineStyleOrder',{'-',':','--','-.'})
plot(0:1:length(files)-3,Magnitude100out,'k',0:1:length(files)-
3,Magnitude10kout,'r') % plot magnitude with semilog scale
xlabel('Time (Hours)')
ylabel('Magnitude (Ohms)')
set(gca,'YScale','log')
set(gcf,'Color','w')
xlim([0 length(files)-3])
ylim([60000 4000000])                      % set Y axis limits
figure(2)                                  % Make Figure 2 active
plot(0:1:length(files)-3,Phase100out,'k',0:1:length(files)-
3,Phase10kout,'r')
```

```
hold on
xlabel('Time (Hours)')
ylabel('Phase (Degrees)')
set(gcf,'Color','w')
ylim([-65 0])
xlim([0 length(files)-3])
cd(HomeDirectory)
```

Appendix D

```
% Impedance plotter
% Ross Wendell 4-7-2009
%
% This script parses all the impedance data files in a directory and
plots
% magnitude and phase as a function of time.
% Start:100 Stop:10000 #Points:150

clear all                                                % Housekeeping
clc
figure(1)
clf
figure(2)
clf

HomeDirectory = 'I:\';                                   % MATLAB Home
Directory
DataDirectory = input('Data directory?\n','s');          % Query for data file
subdirectory
FileExtension = '.txt';                                  % Extension used for
data files
delimiter = '\t';                                        % data file delimiter
R=0;                                                     % start row
C=1;                                                     % start column
t=0;

cd(HomeDirectory)                          % Make sure that we are in the
home directory
cd(DataDirectory)                          % Navigate to the directory of
data files
files = dir;                               % Retrieve a structured list of
filenames
for m = 1:length(files)                    % Main loop - cycle through all
the files
    filename = files(m).name;              % Extract the current filename
from the list
    if isempty(strfind(filename,FileExtension)) % Is it a text file?
        continue                           % If not, skip it.
    end
    try
        impdata = dlmread(filename, delimiter, R, C);     % Read in a
matrix of data from the file
    catch ME
        continue                           % If that didn't work, skip the
file
    end
    N = length(impdata);
    if N < 150                              % Is the file too short to
contain data?
```

```
        OutStr = [10 filename ' is not a full data set']% If so, note
that fact
        continue                            % Don't try to process the file,
and move on
    end
    freq = impdata(:,1);                    % Extract the frequency data
    Magnitude = impdata(:,2);               % Extract the magnitude data
    Phase = impdata(:,3);                   % Extract the phase data
    L = length(files);
    time = t*ones(1,150);
    figure(1)                               % Make Figure 1 active figure
    set(gca,'YDir','reverse')
    set(figure(1),'Position',[10,10,1260,940])     %set position
    xlim([0 L])
    xlabel('time (hours)')
    ylabel('frequency (Hz)')
    zlabel('impedance (ohms)')
    set(gca,'YScale','log','ZScale','log')
    set(gcf,'Color','w')
    plot3(time,freq,Magnitude)              % plot magnitude with semilog
scale
    set(gca,'YDir','reverse')
    zlim([100000 8000000])                  % set Y axis limits
    xlim([0 L])
    hold on                                 % plot all curves together
    figure(2)                               % Make Figure 2 active
    set(gca,'YDir','reverse')
    set(figure(2),'Position',[1290,10,1260,940])
    xlabel('time (hours)')
    ylabel('frequency (Hz)')
    zlabel('phase (degrees)')
    set(gca,'YScale','log')
    set(gcf,'Color','w')
    plot3(time,freq,Phase)
    set(gca,'YDir','reverse')
    zlim([-65 0])
    xlim([0 L])
    hold on
    t=t+1;
end
cd(HomeDirectory)
```